

1724-Pos Board B454**The Role of the Fifth Subunit on Sensitivity of $\alpha 4\beta 2$ -Containing Nicotinic Acetylcholine Receptors to Allosteric Modulators**

Constanza Alcaino.

Oxford Brookes University, Oxford, United Kingdom.

$\alpha 4\beta 2$ nicotinic acetylcholine receptors (nAChRs) belong to the family of pentameric ligand gated ion channels, which includes muscle nAChRs, 5-HT₃, GABA receptors type A and C, and glycine receptors. $\alpha 4\beta 2$ nAChRs are the predominant heteromeric nAChR in the brain. The $\alpha 4\beta 2$ nAChR is the predominant nAChR subtype in the brain. Here, it constitutes one of the most important modulatory receptor systems influencing activities such as cognition, mood, consciousness and nociception and converging evidence indicates that this type is a key mediator of the rewarding and reinforcing effects of nicotine. $\alpha 4$ and $\beta 2$ nAChR subunits assemble into alternate stoichiometries ($\alpha 4\beta 2$)₂ $\alpha 4$ and ($\alpha 4\beta 2$)₂ $\beta 2$ that display remarkable differing sensitivity to activation by agonists and allosteric modulators (Moroni et al., 2006; Carbone et al., 2009). Positive allosteric modulators of $\alpha 4\beta 2$ nAChRs have attracted considerable interest as potential tools for the treatment of cognitive impairment, chronic pain and depression, and as an aid for smoking cessation. However, despite the therapeutic potential of these types of compounds, it is not yet known the structural elements determining stoichiometry-specific sensitivity to $\alpha 4\beta 2$ nAChR allosteric modulators. Here, we have used fully concatenated ($\alpha 4\beta 2$)₂ $\alpha 4$, ($\alpha 4\beta 2$)₂ $\beta 2$ and ($\alpha 4\beta 2$)₂ $\alpha 5$ nAChRs in combination with chimeric receptors, single point mutations and homology models of the alternate $\alpha 4\beta 2$ nAChRs to determine the effects of desformylflustrabromine (dFBr), a selective positive allosteric modulator of $\alpha 4\beta 2$ nAChR, on $\alpha 4\beta 2$ nAChRs and structural elements responsible for those effects. We show that dFBr effects are receptor stoichiometry-dependent and that the fifth subunits is a major determinant of the potentiating effects of dFBr.

1725-Pos Board B455**An Integrative Model of the Pleiotropic Effects of Genistein and Cyclosporine A in a Functional Upregulation of Alpha 7 nAChRs**Joseph Farley¹, Mohammad Faridul Islam¹, Patrick Beard Schwartz¹, Kristi DeBoeuf¹, Jed Rose².

¹Neuroscience, Indiana University, Bloomington, IN, USA, ²Department of Psychiatry and Behavioral Sci., Duke University Med School, Durham, NC, USA.

We have identified several distinct mechanisms by which inhibitors of tyrosine kinases (genistein) and calcineurin/PP2B (cyclosporine A, CsA) produce functional upregulation of $\alpha 7$ Rs expressed in *Xenopus* oocytes. These effects varied with the duration of drug exposure and time course of expression. Genistein's potentiation of peak currents was greater early in expression (2 vs. 5 days following $\alpha 7$ -cRNA injection), and for prolonged drug exposures (24 hr vs 1 min). CsA's potentiation was limited to 24 hr exposure and was independent of expression time. When combined 5-days post-injection, genistein and CsA produced a facilitation greater than either alone: 5-6 fold for CsA + 1 min genistein vs. 2.6-fold for CsA and 2.3-fold for 1 min genistein; 8.5 fold for CsA + 24 hr genistein vs. 2.6 fold for CsA and 4.3 fold for 24 hr genistein. Similarly, 2-days following cRNA injections, 24 hr CsA plus 1 min genistein produced facilitation greater than either alone: 4.5 fold vs. 1.5 for CsA and 2.8 for genistein. However, the combination of CsA and genistein (24 hr) produced facilitation (5.3 fold) that was less than genistein alone: 1.5 for CsA, and 12 for genistein. We propose a model to explain this pattern that assumes: 1) 1 min genistein's effects are exclusively PAM, while 24 hr genistein's effects are PAM and non-PAM (all effects are positive), 2) the effects of CsA alone are net positive, but reflect two opposing processes. CsA facilitates currents by increasing surface membrane numbers of $\alpha 7$ -Rs and/or single-channel gating. But CsA also inhibits cRNA-translation and/or chaperoning of $\alpha 7$ -Rs. The inhibitory effect is greater early in expression, reducing the number of functional $\alpha 7$ -Rs distal to the ER/Golgi pathway that genistein can positively interact with.

1726-Pos Board B456**Structure Function Studies at Two Different Nicotinic Acetylcholine Receptor Subtypes**Michael R. Post¹, Henry A. Lester², Dennis A. Dougherty¹.

¹Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA, USA, ²Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA. The nicotinic acetylcholine receptor (nAChR) is involved with regulating the release of neurotransmitter in the synapse. This research focuses on two sub-

types of neuronal nAChR, the $\alpha 6\beta 2$ and $\alpha 4\beta 2$ receptors. Subtypes containing the $\alpha 6$ and $\beta 2$ subunits are found mainly in dopaminergic neurons. The acetylcholine binding site in these subtypes, which sits at the interface of the two subunits, is an important therapeutic target. Expression and activation of a pure population of $\alpha 6\beta 2$ receptors in *X. laevis* oocytes has previously never been reported. This study used two mutations in the $\beta 2$ M3-M4 loop and a reporter mutation in the M2 helix of each subunit to successfully express $\alpha 6\beta 2$ receptors. Expression has been optimized, and a method to control subunit stoichiometry has been developed. We then used unnatural amino acid mutagenesis to develop a binding model for acetylcholine and nicotine at the $\alpha 6$ - $\beta 2$ subunit interface.

The $\alpha 4\beta 2$ subtype is the most prevalent nAChR subtype found in the brain and is implicated in many neurological disorders. Previous work has established a hydrogen bond interaction between the pyrrolidine nitrogen of nicotine and the carbonyl backbone adjacent to Trp149 in the $\alpha 4$ subunit. N⁷-methylnicotinium, which has a quaternary amine at the pyrrolidine nitrogen and cannot donate a hydrogen bond, was synthesized in order to probe this hydrogen bond donating interaction. Through a double-mutant cycle analysis utilizing an α -hydroxy acid mutation to perturb the carbonyl backbone hydrogen bond acceptor, we have determined that the hydrogen bonding interaction has a coupling energy of ~2 kcal/mol.

1727-Pos Board B457**Identifying Motifs Essential for Selective Activation of Nicotinic Acetylcholine Receptors**Christopher Marotta¹, Henry A. Lester², Dennis A. Dougherty¹.

¹Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA, USA, ²Department of Biology, California Institute of Technology, Pasadena, CA, USA.

Neuronal Nicotinic acetylcholine receptors (nAChRs) are found throughout the brain and have vital roles in memory and learning. In addition, these ligand gated ion channel are major targets of drug research for neurological disorders. Here, the two stoichiometries of $\alpha 4\beta 2$ nAChRs expressed in *Xenopus* oocytes were probed using two agonists that display stoichiometry selectivity: Sazetidine A and NS9283. The results from these studies led to the following conclusions that may be generalized to the nAChR family: 1) an agonist must be bound to each α subunit in the receptor in order to fully activate the receptor and 2) three key residues located on the complementary side of the subunit dictate agonist selectivity. Through better understanding of agonist binding, more potent and selective responses with minimized off target responses can be obtained.

1728-Pos Board B458**Insights into the Distinctly Different Sensitivities of $\alpha 7$ and $\alpha 7\beta 2$ nAChRs to the Volatile Anesthetic Isoflurane**David D. Mowrey¹, Qiang Liu², Vasyli Bondarenko³, Qiang Chen³, Edom Seyoum³, Yan Xu³, Jie Wu², Pei Tang³.

¹Computational Biology, University of Pittsburgh, Pittsburgh, PA, USA, ²Division of Neurology, Barrow Neurological Institute, St Joseph's Hospital and Medical Center, Phoenix, AZ, USA, ³Anesthesiology, University of Pittsburgh, Pittsburgh, PA, USA.

Nicotinic acetylcholine receptors (nAChRs) mediate the fast synaptic transmission in the central and peripheral nervous systems. They are also targets of general anesthetics. Among the nAChR subtypes, $\alpha 7$ and $\alpha 4\beta 2$ nAChRs are the most abundant subtypes found in the brain. Despite their high sequence homology, the $\alpha 4\beta 2$ nAChR is hypersensitive but the $\alpha 7$ nAChR is insensitive to volatile general anesthetics. The underlying cause for the functional discrepancy between these homologous receptors presents an intriguing enigma. In this study, we have found that the $\alpha 7\beta 2$ nAChR is hypersensitive to the volatile anesthetic isoflurane and that the $\beta 2$ subunit is primarily responsible for conferring anesthetic sensitivity. Using high-resolution NMR, we identified intrasubunit isoflurane binding sites at the extracellular and intracellular ends of the $\beta 2$ and $\alpha 7$ transmembrane domains, respectively. Cavity analyses revealed that $\beta 2$, but not $\alpha 7$, has a pocket large enough to accommodate isoflurane binding to the extracellular end of the transmembrane domain. Variations in pocket size result from subtle structural differences between $\beta 2$ and $\alpha 7$, where the angle between the TM2 and TM4 helices differs by ~7° between $\beta 2$ and $\alpha 7$. While isoflurane binding perturbed dynamics for both $\beta 2$ and $\alpha 7$, only the binding to the extracellular end of the $\beta 2$ transmembrane domain produced significant dynamics changes for the pore-lining residues. The study suggests that a subtle structural difference could affect drug binding. Furthermore, only the drug binding that affects the dynamics of the channel gate is able to generate the functional effect. Supported by NIH (R01GM66358, R01GM56257, and R37GM049202).